Detoxification of LPS by alkaline phosphatase

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Chapter 1

Aim and outline of the thesis

Alkaline phosphatase (AP) is an endogenous enzyme present in many organs of the human body. The intestine, liver and kidney and also placenta are all organs in which AP is especially abundantly present. However, since AP is also expressed in blood cells such as neutrophils and vascular cells like endothelial cells, the enzyme in fact is active everywhere in the human body. In addition, AP is also present in bone tissue and plays a crucial role in the formation of bone material (1). Another and more recently recognized function of AP is its role as a lipopoly-saccharide(LPS)-detoxifying enzyme. This function of AP was first established by Poelstra *et al* in 1997 (2; 3).

In diseases associated with elevated serum LPS levels, like sepsis, liver diseases like fibrosis, cholestasis, hepatitis B & C and obstructive jaundice as well as inflammatory bowel diseases like Crohn's disease (CD) and ulcerative colitis (UC), AP levels are usually changed compared to the normal situation. AP levels in serum and/or tissues are elevated or decreased. Yet, the mechanism for its regulation and the cause of the abnormal levels are in many cases unclear. Since abnormal AP levels are seen in most, if not all, LPS-associated diseases and AP is capable of detoxifying LPS, the question arises how AP tissue expression is regulated and whether AP might be a natural part of the immune system by serving as defense system against LPS-bearing Gramnegative bacteria. If it has a protective role, exogenous administration of AP or upregulation of endogenous AP activity during these diseases, in which LPS is an important pathogenic factor, could exert beneficial effects.

The work described in this thesis can grossly be divided into two themes. The first theme deals with the regulation of endogenous liver AP (Ch. 3 and 4) and the second theme focuses on the possible application of exogenous AP in LPS-mediated diseases (Ch. 3, 5 and 6).

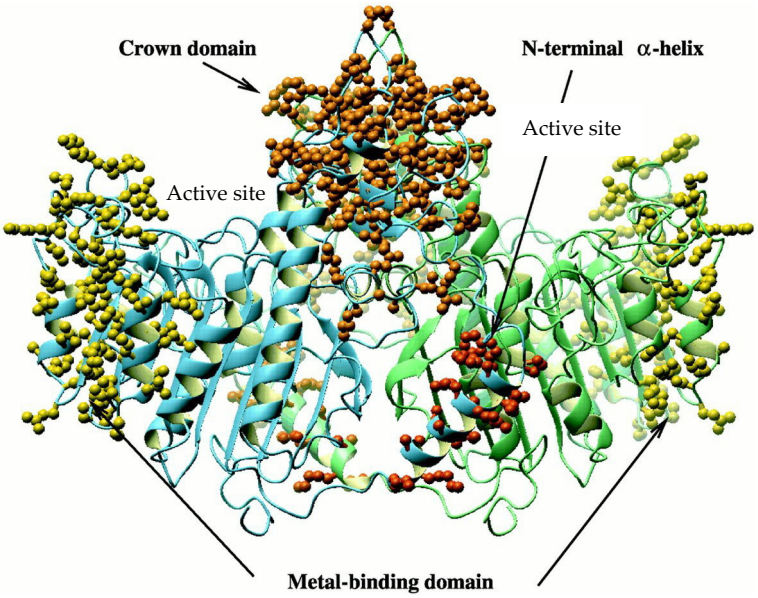

Figure 1: Structure of the enzyme alkaline phosphatase. The two monomers are shown in blue (left) and green (right). The two metal binding domains are shown in yellow, the crown domain in brown and the location of both active sites is indicated. From Le Du, 2001 (a full color version of this figure can be found on page 169).

Chapter 3 contains a report on the LPS-dephosphorylating activity in the rat liver. Although it is known that the liver is the major organ in the body that removes LPS from the circulation, very little is known about the capability of the liver itself to dephosphorylate LPS. We investigated the LPS-dephosphorylating activity of normal and fibrotic rat livers histochemically using several serotypes of LPS. Liver fibrosis was shown to be associated with altered AP levels and altered LPS responsiveness. Furthermore, we explored the effect of LPS on AP levels in rat livers *in vivo* and examined the effects of administration of intestinal AP to rats with LPS-induced sepsis.

In the experiments described in **chapter 4**, we studied the effect of LPS on liver AP expression in liver cell lines, liver slices and *in vivo* in rats. The liver cell lines HepG2 and McA-Rh7777 were incubated with LPS and the well-known AP-inducers retinoic acid and sodium butyrate. Also freshly prepared liver slices were

stimulated with LPS and the effects on AP expression were examined. In addition, expression levels of several cytokines were measured to gain more insight in the possible regulation of liver AP expression. Finally, liver AP expression was examined *in vivo* at several timepoints after LPS administration in rats.

Chapter 5 describes the effects of 2 AP isoenzymes, placental AP and intestinal AP, on sepsis in mice. In addition, in this chapter we also introduced a novel assay for the detection of LPS. In this new assay, the dicationic molecule pentamidine is used, which fluoresces upon binding to LPS via its diphosphorylated lipid A part, but not with the monophosphorylated lipid A part.

The possible role of intestinal AP (iAP) as a therapeutic enzyme in inflammatory bowel disease (IBD) has been examined in the final research chapter, **chapter 6**. Effects of iAP on sepsis were described in several models of sepsis in mice (4; 5) and pigs (6) while clinical trials in humans are ongoing. So far, there are no reports about the use of AP in IBD, although several lines of research indicate a role for LPS in this disease (7). We now assessed the effect of iAP on LPS-stimulated human biopsies of healthy persons and patients suffering from CD or UC. Moreover, we studied the effect of oral administration of iAP in tablet form on the progression of dextran sodium sulphate-induced colitis in rats.

In summary, the studies described in this thesis examined factors that regulate AP activity *in vivo* (Ch. 3 and 4) and the potential applicability of exogenous AP in LPS mediated diseases like sepsis (Ch. 3 and 5) as well as inflammatory bowel disease (Ch. 6). Also the possibility to modify and selectively target AP to pathogenic cells was explored.

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